

RECEIVED

OCT 24 2002

TECH CENTER 1600/2900

SEQUENCE LISTING

<110> Meares, Claude
Chmura, Albert
The Regents of the University of California

<120> Engineering Antibodies That Bind Irreversibly

<130> 023070-099120US

<140> US 09/671,953
<141> 2000-09-27

<150> US 60/156,194
<151> 1999-09-27

<150> US 60/208,684
<151> 2000-05-31

<160> 23

<170> PatentIn Ver. 2.1

<210> 1
<211> 753
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:nucleic acid
that encodes Fab heavy chain of CHA255

<400> 1
agatctgaag tgacgctgg ggagtctagg ggagactcag tgaagcctgg agggttcctg 60
aaactctcct gtgcagcctc tggattcaact ttaagtgggt aaaccatgtc ttgggttcgc 120
cagactccgg agaagaggct ggagtgggtc acaaccactc tttagtgggttgg tggtttcacc 180
ttctatttcag ccagtgtgaa gggtcgttcc accatctcca gagacaatgc ccagaacaac 240
ctctatctac aactgaatag tctgaggctt gaggacacgg ccttgatatt ctgtgcaagt 300
catcggtttt ttcactgggg ccacgggact ctgtcactg tctctgcagc caaaacgacg 360
ggcccatcggtt tcttccccctt ggcacccctcc tccaaagagca cctctggggg cacagcggcc 420
ctgggctgcc tggtaagga ctacttcccc gaaccgggtga cgggtgtcgta gaactcagggc 480
gccctgacca gcggcgtgca caccttccc gctgtcctac agtcctcaag actctacttc 540
ctcagcagcg tggtgaccgt gcccctcaac agcttgggca cccagaccta catctgcaac 600
gtgaatcaca agcccagcaa caccagggtg gacaagaaaag cagagccaa atcttgtgac 660
aaatctagag ggccttcga aggttaaggct atccctaacc ctctcctcgg ttcgtattct 720
acgcgttaccg gtcatcatca ccatcaccat tga 753 .

<210> 2
<211> 657
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:nucleic acid
that encodes light chain mutant with Cys
substituted for Asn at position 97 of CHA255

<400> 2
agatctgctg ttgtgactca ggaatctgca ctcaccacat cacctgggtga aacagtccaca 60
ctcacttgc gctcaagtat tggggctgtt acaactagta actatgccaa ctgggtccaa 120

gaaaaaccag atcatttatt cactggtcta ataggtggta ccaataaccg ggctccgggt 180
gtcctgcca gatttcagg ctccctgatt ggagacaagg ctgcctcac catcacaggg 240
gcacagactg aagatgaggc aagatatttc tggctctat ggtactcctg cctctgggr 300
ttcggtgag gaaccaaact gactgtccta agccgwackg tggctgcacc atctgtcttc 360
atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420
aataacttct atcccagaga ggccaaagta cagtgaaagg tggataaacgc cctccaatcg 480
ggttaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540
agcaccctga cgctgagcaa agcagactac gaaaaacaca aagtctacgc ctgcgaagtc 600
accatcagg gcctgagty gcccgtcaca aagagttca acaggggaga gtgttaa 657

<210> 3
<211> 657
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:nucleic acid
that encodes the unmodified light chain of CHA255

<400> 3
agatctgctg ttgtgactca ggaatctgca ctcaccacat cacctggta aacagtcaca 60
ctcacttgc gctcaagtat tggggctgtt acaacttagta actatgccaa ctgggtccaa 120
gaaaaaccag atcatttatt cactggtcta ataggtggta ccaataaccg ggctccgggt 180
gtcctgcca gatttcagg ctccctgatt ggagacaagg ctgcctcac catcacaggg 240
gcacagactg aagatgaggc aagatatttc tggctctat ggtactcctg cctctgggr 300
ttcggtgag gaaccaaact gactgtccta agccgwackg tggctgcacc atctgtcttc 360
atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420
aataacttct atcccagaga ggccaaagta cagtgaaagg tggataaacgc cctccaatcg 480
ggttaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540
agcaccctga cgctgagcaa agcagactac gaaaaacaca aagtctacgc ctgcgaagtc 600
accatcagg gcctgagty gcccgtcaca aagagttca acaggggaga gtgttaa 657

<210> 4
<211> 657
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:nucleic acid
that encodes light chain mutant with Cys
substituted for Ser at position 96 of CHA255

<400> 4
agatctgctg ttgtgactca ggaatctgca ctcaccacat cacctggta aacagtcaca 60
ctcacttgc gctcaagtat tggggctgtt acaacttagta actatgccaa ctgggtccaa 120
gaaaaaccag atcatttatt cactggtcta ataggtggta ccaataaccg ggctccgggt 180
gtcctgcca gatttcagg ctccctgatt ggagacaagg ctgcctcac catcacaggg 240
gcacagactg aagatgaggc aagatatttc tggctctat ggtactgcaa cctctgggr 300
ttcggtgag gaaccaaact gactgtccta agccgwackg tggctgcacc atctgtcttc 360
atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420
aataacttct atcccagaga ggccaaagta cagtgaaagg tggataaacgc cctccaatcg 480
ggttaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540
agcaccctga cgctgagcaa agcagactac gaaaaacaca aagtctacgc ctgcgaagtc 600
accatcagg gcctgagty gcccgtcaca aagagttca acaggggaga gtgttaa 657

<210> 5
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: polypeptide sequence of mutant light chain with Cys substituted for Asn at position 97 of CHA255

<220>
<221> MOD_RES
<222> (207)
<223> Xaa = any amino acid

<400> 5
Arg Ser Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly
1 5 10 15

Glu Thr Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr
20 25 30

Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr
35 40 45

Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg
50 55 60

Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly
65 70 75 80

Ala Gln Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95

Cys Leu Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Ser Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Xaa Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 6
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: polypeptide
sequence of unmodified light chain of CHA255

<220>
<221> MOD_RES
<222> (207)
<223> Xaa = any amino acid

<400> 6
Arg Ser Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly
1 5 10 15

Glu Thr Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr
20 25 30

Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr
35 40 45

Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg
50 55 60

Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly
65 70 75 80

Ala Gln Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95

Asn Leu Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Ser Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Xaa Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 7
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: polypeptide sequence of mutant light chain with Cys substituted for Ser at position 96 of CHA255

<220>
<221> MOD_RES
<222> (207)
<223> Xaa = any amino acid

<400> 7
Arg Ser Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly
1 5 10 15

Glu Thr Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr
20 25 30

Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr
35 40 45

Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg
50 55 60

Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly
65 70 75 80

Ala Gln Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys
85 90 95

Asn Leu Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Ser Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Xaa Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 8
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: polypeptide sequence of unmodified heavy chain of CHA255

<400> 8
Arg Ser Glu Val Thr Leu Val Glu Ser Arg Gly Asp Ser Val Lys Pro
1 5 10 15

Gly Gly Phe Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser
20 25 30

Gly Glu Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu
35 40 45

Trp Val Thr Thr Leu Ser Gly Gly Phe Thr Phe Tyr Ser Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Asn
65 70 75 80

Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr
85 90 95

Phe Cys Ala Ser His Arg Phe Val His Trp Gly His Gly Thr Leu Val
100 105 110

Thr Val Ser Ala Ala Lys Thr Thr Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Arg Leu Tyr Phe Leu Ser Ser Val Val Thr Val Pro Phe Asn Ser Leu
180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195 200 205

Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Ser Arg Gly
210 215 220

Pro Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser
225 230 235 240

Thr Arg Thr Gly His His His His His His
245 250

```

<210> 9
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:T7 promoter
      primer

<400> 9
ctataacgac tcactatagg g 21

<210> 10
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:K XbaI primer

<400> 10
ctgcaggtcg actcttagagg atctactagt 30

<210> 11
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:mutagenic site

<400> 11
catgcctgca ggtcgactct agaggatcta ctagt 35

<210> 12
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:mutagenic site

<400> 12
ttctgtgctc tatggtagac caacctctgg gtattcggt 39

<210> 13
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:mutagenesis
      primer S95C

<400> 13
atacccagag gttgcagtag catagagcac 30

```

```

<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:U-19 primer

<400> 14
ggtttccca gtcacgacg 19

<210> 15
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:mutagenesis
      primer N96C

<400> 15
ataccaggag gcagctgtac catagagcac 30

<210> 16
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:V-H sequence of
      CHA255

<220>
<221> CDS
<222> (1)..(360)

<400> 16
gaa gtg acg ctg gtg gag tct ggg gga gac tca gtg aag cct gga ggg 48
Glu Val Thr Leu Val Glu Ser Gly Gly Asp Ser Val Lys Pro Gly Gly
   1           5               10             15

tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc act tta agt ggt gaa 96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser Gly Glu
   20          25               30

acc atg tct tgg gtt cgc cag act ccg gag aag agg ctg gag tgg gtc 144
Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
   35          40               45

gca acc act ctt agt ggt ggt ttc acc ttc tat tca gcc agt gtg 192
Ala Thr Thr Leu Ser Gly Gly Phe Thr Phe Tyr Ser Ala Ser Val
   50          55               60

aag ggt cgt ttc acc atc tcc aga gac aat gcc cag aac aac ctc tat 240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Asn Leu Tyr
   65          70               75             80

```

cta caa ctg aat agt ctg agg tct gag gac acg gcc ttg tat ttc tgt 288
Leu Gln Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Phe Cys
85 90 95

gca agt cat cgg ttt gtt cac tgg ggc cac ggg act ctg gtc act gtc 336
Ala Ser His Arg Phe Val His Trp Gly His Gly Thr Leu Val Thr Val
100 105 110

tct gca gcc aaa acg aca ccc cca 360
Ser Ala Ala Lys Thr Thr Pro Pro
115 120

<210> 17
<211> 120
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:V-H sequence of
CHA255

<400> 17
Glu Val Thr Leu Val Glu Ser Gly Gly Asp Ser Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser Gly Glu
20 25 30

Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Thr Leu Ser Gly Gly Phe Thr Phe Tyr Ser Ala Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Asn Leu Tyr
65 70 75 80

Leu Gln Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Phe Cys
85 90 95

Ala Ser His Arg Phe Val His Trp Gly His Gly Thr Leu Val Thr Val
100 105 110

Ser Ala Ala Lys Thr Thr Pro Pro
115 120

<210> 18
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:cloning primer
with XhoI site

<400> 18
ggtgctcgag tctggggag actcagtg 28

```

<210> 19
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:cloning primer
      with ApaI site

<400> 19
ggaggggcccg tcgtttggc tgcaga                                26

<210> 20
<211> 405
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:V-L sequence of
      CHA255 mutant S95C

<220>
<221> CDS
<222> (1)..(405)

<220>
<221> modified_base
<222> (405)
<223> n = g, a, c or t

<400> 20
gct gtt gtg act cag gaa tct gca ctc acc aca tca cct ggt gaa aca    48
Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr
   1           5           10          15

gtc aca ctc act tgt cgc tca agt att ggg gct gtt aca act agt aac    96
Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr Ser Asn
   20          25          30

tat gcc aac tgg gtc caa gaa aaa cca gat cat tta ttc act ggt cta    144
Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu
   35          40          45

ata ggt ggt acc aat aac cgg gct ccg ggt gtt cct gcc aga ttc tca    192
Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg Phe Ser
   50          55          60

ggc tcc ctg att gga gac aag gct gcc ctc acc atc aca ggg gca cag    240
Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln
   65          70          75          80

act gaa gat gag gca aga tat ttc tgt gct cta tgg tac tgc aac ctc    288
Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys Asn Leu
   85          90          95

tgg gtg ttc ggt gga gga acc aaa ctg act gtc cta agc cag ccc aag    336
Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Ser Gln Pro Lys
   100         105         110

```

tct tcg cca tca gtc acc ctg ttt ccg ccc tcc tct gaa gag cta agc 384
Ser Ser Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Ser
115 120 125

ttg gga atc gga ttc ccg ggn 405
Leu Gly Ile Gly Phe Pro Gly
130 135

<210> 21
<211> 135
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:V-L sequence of
CHA255 mutant S95C

<400> 21
Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr
1 5 10 15

Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr Ser Asn
20 25 30

Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu
35 40 45

Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg Phe Ser
50 55 60

Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln
65 70 75 80

Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys Asn Leu
85 90 95

Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Ser Gln Pro Lys
100 105 110

Ser Ser Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Ser
115 120 125

Leu Gly Ile Gly Phe Pro Gly
130 135

<210> 22
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:cloning primer
with SstI site

<400> 22
ctcagagctc gctgttgtga ctcaggaatc t 31

<210> 23
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:cloning primer
with BsiWI site

<400> 23
ctcgcatgcg cttaggacag tcagttt

27